AVMA Guidelines on Euthanasia

(Formerly Report of the AVMA Panel on Euthanasia)

June 2007

Caution - The AVMA Guidelines on Euthanasia (formerly the 2000 Report of the AVMA Panel on Euthanasia) have been widely misinterpreted. Please note the following:

- The guidelines are in no way intended to be used for human lethal injection.
- The application of a barbiturate, paralyzing agent, and potassium chloride delivered in separate syringes or stages (the common method used for human lethal injection) is not cited in the report.
- The report never mentions pancuronium bromide or Pavulon, the paralyzing agent used in human lethal injection.

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PREFACE

At the request of the American Veterinary Medical Association's (AVMA) Council on Research, the Executive Board of the AVMA convened a Panel on Euthanasia in 1999 to review and make necessary revisions to the fifth Panel Report, published in 1993.[1] The Report of the 2000 AVMA Panel on Euthanasia was published in the *Journal of the American Veterinary Medical Association*.[216] In that version of the report, the panel updated information on euthanasia of animals in research and animal care and control facilities; expanded information on ectothermic, aquatic, and fur-bearing animals; added information on horses and wildlife; and deleted methods or agents considered unacceptable. Because the panel's deliberations were based on currently available scientific information, some euthanasia methods and agents are not discussed.

In 2006, the AVMA Executive Board approved a recommendation that the AVMA convene a panel of scientists at least once every 10 years to review all literature that scientifically evaluates methods and potential methods of euthanasia for the purpose of producing AVMA Guidelines on Euthanasia. During interim years, requests for inclusion of new or altered euthanasia procedures or agents in the AVMA Guidelines on Euthanasia are directed to the AVMA Animal Welfare Committee (AWC). Revisions are based on a thorough evaluation of the available science and require Executive Board approval. The first interim revision, approved in 2006, is the addition of a physical method (maceration) for euthanasia of chicks, poults, and pipped eggs. Substantive interim additions in the Guidelines are indicated by text that is underlined.

Welfare issues are increasingly being identified in the management of free-ranging wildlife, and the need for humane euthanasia guidelines in this context is great. Collection of animals for scientific investigations, euthanasia of injured or diseased wildlife species, removal of animals causing damage to property or threatening human safety, and euthanasia of animals in excess population are drawing more public attention. These issues are acknowledged in these guidelines and special considerations are described for handling animals under free-ranging conditions, where their needs are far different from those of their domestic counterparts.

These guidelines are intended for use by members of the veterinary profession who carry out or oversee the euthanasia of animals. Although the guidelines may be interpreted and understood by a broad segment of the general population, a veterinarian should be consulted in the application of these recommendations. The practice of veterinary medicine is complex and involves diverse animal species. Whenever possible, a veterinarian experienced with the species in question should be consulted when selecting the method of euthanasia, particularly when little species-specific euthanasia research has been done. Although interpretation and use of these guidelines cannot be limited, the AVMA's overriding commitment is to give veterinarians guidance in relieving pain and suffering of animals that are to be euthanatized. The recommendations in these guidelines are intended to serve as guidance for veterinarians who must then use professional judgment in applying them to the various settings where animals are to be euthanatized.

INTRODUCTION

The term euthanasia is derived from the Greek terms *eu* meaning good and *thanatos* meaning death.[2] A "good death" would be one that occurs with minimal pain and distress. In the context of these guidelines, euthanasia is the act of inducing humane death in an animal. It is our responsibility as veterinarians and human beings to ensure that if an animal's life is to be taken, it is done with the highest degree of respect, and with an emphasis on making the death as painless and distress free as possible. Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function. In addition, the technique should minimize distress and anxiety experienced by the animal prior to loss of consciousness. The panel recognized that the absence of pain and distress cannot always be achieved. These guidelines attempt to balance the ideal of minimal pain and distress with the reality of the many environments in which euthanasia is performed. A veterinarian with appropriate training and expertise for the species involved should be consulted to ensure that proper procedures are used.

Criteria for painless death can be established only after the mechanisms of pain are understood. Pain is that sensation (perception) that results from nerve impulses reaching the cerebral cortex via ascending neural pathways. Under normal circumstances, these pathways are relatively specific, but the nervous system is sufficiently plastic that activation of nociceptive pathways does not always result in pain and stimulation

of other (non-nociceptive) peripheral and central neurons can give rise to pain. The term nociceptive is derived from the word *noci* meaning to injure and *ceptive* meaning to receive, and is used to describe neuronal input caused by noxious stimuli, which threaten to, or actually do, destroy tissue. These noxious stimuli initiate nerve impulses by acting at primary nociceptors and other sensory nerve endings that respond to noxious and non-noxious stimuli from mechanical, thermal, or chemical activity. Endogenous chemical substances such as hydrogen ions, potassium ions, ATP, serotonin, histamine, bradykinin, and prostaglandins, as well as electrical currents, are capable of generating nerve impulses in nociceptor nerve fibers. Activity in nociceptive pathways can also be triggered in normally silent receptors that become sensitized by chronic pain conditions.[3,4]

Nerve impulse activity generated by nociceptors is conducted via nociceptor primary afferent fibers to the spinal cord or the brainstem where it is transmitted to two general sets of neural networks. One set is related to nociceptive reflexes (eg, withdrawal and flexion reflexes) that are mediated at the spinal level, and the second set consists of ascending pathways to the reticular formation, hypothalamus, thalamus, and cerebral cortex (somatosensory cortex and limbic system) for sensory processing. It is important to understand that ascending nociceptive pathways are numerous, often redundant, and are capable of considerable plasticity under chronic conditions (pathology or injury). Moreover, even the transmission of nociceptive neural activity in a given pathway is highly variable. Under certain conditions, both the nociceptive reflexes and the ascending pathways may be suppressed, as, for example, in epidural anesthesia. Under another set of conditions, nociceptive reflex actions may occur, but activity in the ascending pathways is suppressed; thus, noxious stimuli are not perceived as pain. It is incorrect to use the term pain for stimuli, receptors, reflexes, or pathways because the term implies perception, whereas all the above may be active without consequential pain perception.[5,6]

Pain is divided into two broad categories: (1) sensory-discriminative, which indicates the site of origin and the stimulus giving rise to the pain; and (2) motivational-affective in which the severity of the stimulus is perceived and the animal's response is determined. Sensory-discriminative processing of nociceptive impulses is most likely to be accomplished by subcortical and cortical mechanisms similar to those used for processing other sensory-discriminative input that provides the individual with information about the intensity, duration, location, and quality of the stimulus. Motivational-affective processing involves the ascending reticular formation for behavioral and cortical arousal. It also involves thalamic input to the forebrain and the limbic system for perceptions such as discomfort, fear, anxiety, and depression. The motivational-affective neural networks also have strong inputs to the limbic system, hypothalamus and the autonomic nervous system for reflex activation of the cardiovascular, pulmonary, and pituitary-adrenal systems. Responses activated by these systems feed back to the forebrain and enhance perceptions derived via motivational-affective inputs. On the basis of neurosurgical experience in humans, it is possible to separate the sensory-discriminative components from the motivational-affective components of pain.[7]

For pain to be experienced, the cerebral cortex and subcortical structures must be functional. If the cerebral cortex is nonfunctional because of hypoxia, depression by drugs, electric shock, or concussion, pain is not experienced. Therefore, the choice of the euthanasia agent or method is less critical if it is to be used on an animal that is anesthetized or unconscious, provided that the animal does not regain consciousness prior to death.

An understanding of the continuum that represents stress and distress is essential for evaluating techniques that minimize any distress experienced by an animal being euthanatized. Stress has been defined as the effect of physical, physiologic, or emotional factors (stressors) that induce an alteration in an animal's homeostasis or adaptive state.[8] The response of an animal to stress represents the adaptive process that is necessary to restore the baseline mental and physiologic state. These responses may involve changes in an animal's neuroendocrinologic system, autonomic nervous system, and mental status that may result in overt behavioral changes. An animal's response varies according to its experience, age, species, breed, and current physiologic and psychologic state.[9]

Stress and the resulting responses have been divided into three phases.[10] Eustress results when harmless stimuli initiate adaptive responses that are beneficial to the animal. Neutral stress results when the animal's response to stimuli causes neither harmful nor beneficial effects to the animal. Distress results when an animal's response to stimuli interferes with its well-being and comfort.[11]

As with many other procedures involving animals, some methods of euthanasia require physical handling of the animal. The amount of control and kind of

restraint required will be determined by the animal's species, breed, size, state of domestication, degree of taming, presence of painful injury or disease, degree of excitement, and method of euthanasia. Proper handling is vital to minimize pain and distress in animals, to ensure safety of the person performing euthanasia, and, often, to protect other people and animals.

An in-depth discussion of euthanasia procedures is beyond the scope of these guidelines; however, personnel who perform euthanasia must have appropriate certification and training, experience with the techniques to be used, and experience in the humane restraint of the species of animal to be euthanatized, to ensure that animal pain and distress are minimized during euthanasia. Training and experience should include familiarity with the normal behavior of the species being euthanatized, an appreciation of how handling and restraint affects that behavior, and an understanding of the mechanism by which the selected technique induces loss of consciousness and death. Prior to being assigned full responsibility for performing euthanasia, all personnel must have demonstrated proficiency in the use of the technique in a closely supervised environment. References provided at the end of this document may be useful for training personnel.[12-21]

Selection of the most appropriate method of euthanasia in any given situation depends on the species of animal involved, available means of animal restraint, skill of personnel, number of animals, and other considerations. Available information focuses primarily on domestic animals, but the same general considerations should be applied to all species.

These guidelines include four appendices that summarize information from the text. Appendix 1 lists acceptable and conditionally acceptable methods of euthanasia, categorized by species. Appendices 2 and 3 provide summaries of characteristics for acceptable and conditionally acceptable methods of euthanasia. Appendix 4 provides a summary of some unacceptable euthanasia agents and methods. Criteria used for acceptable, conditionally acceptable, and unacceptable methods are as follows: acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia; conditionally acceptable methods are those techniques that by the nature of the technique or because of greater potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in the scientific literature; and unacceptable techniques are those methods deemed inhumane under any conditions or that the panel found posed a substantial risk to the human applying the technique. The guidelines also include discussion of several adjunctive methods, which are those methods that cannot be used as the sole method of euthanasia, but that can be used in conjunction with other methods to produce a humane death.

GENERAL CONSIDERATIONS

In evaluating methods of euthanasia, the panel used the following criteria: (1) ability to induce loss of consciousness and death without causing pain, distress, anxiety, or apprehension; (2) time required to induce loss of consciousness; (3) reliability; (4) safety of personnel; (5) irreversibility; (6) compatibility with requirement and purpose; (7) emotional effect on observers or operators; (8) compatibility with subsequent evaluation, examination, or use of tissue; (9) drug availability and human abuse potential; (10) compatibility with species, age, and health status; (11) ability to maintain equipment in proper working order; and (12) safety for predators/scavengers should the carcass be consumed.

The panel discussed the definition of euthanasia used in these guidelines as it applies to circumstances when the degree of control over the animal makes it difficult to ensure death without pain and distress. Slaughter of animals for food, fur, or fiber may represent such situations. However, the same standards for euthanasia should be applied to the killing of animals for food, fur, or fiber, and wildlife or feral animals. Animals intended for food should be slaughtered humanely, taking into account any special requirements of the US Department of Agriculture.[22] Painless death can be achieved by properly stunning the animal, followed immediately by exsanguination. Handling of animals prior to slaughter should be as stress free as possible. Electric prods or other devices should not be used to encourage movement of animals and are not needed if chutes and ramps are properly designed to enable animals to be moved and restrained without undue stress.[23-27] Animals must not be restrained in a painful position before slaughter.

Ethical considerations that must be addressed when euthanatizing healthy and unwanted animals reflect professional and societal concerns.28,29 These issues are complex and warrant thorough consideration by the profession and all those concerned with the welfare of animals. Whereas the panel

recognized the need for those responsible for the euthanasia of animals to be cognizant of these issues, it did not believe that its report was the appropriate forum for an in-depth discussion of this topic.

It is the intent of AVMA that euthanasia be performed in accordance with applicable federal, state, and local laws governing drug acquisition and storage, occupational safety, and methods used for euthanasia and disposal of animals. However, space does not permit a review of current federal, state, and local regulations.

The panel was aware that circumstances may arise that are not clearly covered by its report. Whenever such situations arise, a veterinarian experienced with the species should use professional judgment and knowledge of clinically acceptable techniques in selecting an appropriate euthanasia technique. Professional judgment in these circumstances will take into consideration the animal's size and its species-specific physiologic and behavioral characteristics. In all circumstances, the euthanasia method should be selected and used with the highest ethical standards and social conscience.

It is imperative that death be verified after euthanasia and before disposal of the animal. An animal in deep narcosis following administration of an injectable or inhalant agent may appear dead, but might eventually recover. Death must be confirmed by examining the animal for cessation of vital signs, and consideration given to the animal species and method of euthanasia when determining the criteria for confirming death.

ANIMAL BEHAVIORAL CONSIDERATIONS

The need to minimize animal distress, including fear, anxiety, and apprehension, must be considered in determining the method of euthanasia. Gentle restraint (preferably in a familiar and safe environment), careful handling, and talking during euthanasia often have a calming effect on animals that are used to being handled. Sedation and/or anesthesia may assist in achieving the best conditions for euthanasia. It must be recognized that any sedatives or anesthetics given at this stage that change circulation may delay the onset of the euthanasia agent. Preparation of observers should also be taken into consideration.

Animals that are wild, feral, injured, or already distressed from disease pose another challenge. Methods of pre-euthanasia handling suitable for domestic animals may not be effective for them. Because handling may stress animals unaccustomed to human contact (eg, wildlife, zoo, and feral species), the degree of restraint required to perform any euthanasia procedure should be considered when evaluating various methods. When handling these animals, calming may be accomplished by minimizing visual, auditory, and tactile stimulation. When struggling during capture or restraint may cause pain, injury, or anxiety to the animal or danger to the operator, the use of tranquilizers, analgesics, and/or anesthetics may be necessary. A route of injection should be chosen that causes the least distress in the animal for which euthanasia must be performed. Various techniques for oral delivery of sedatives to dogs and cats have been described that may be useful under these circumstances.[30,31]

Facial expressions and body postures that indicate various emotional states of animals have been described for some species.[32-37] Behavioral and physiologic responses to noxious stimuli include distress vocalization, struggling, attempts to escape, defensive or redirected aggression, salivation, urination, defecation, evacuation of anal sacs, pupillary dilatation, tachycardia, sweating, and reflex skeletal muscle contractions causing shivering, tremors, or other muscular spasms. Unconscious as well as conscious animals are capable of some of these responses. Fear can cause immobility or "playing dead" in certain species, particularly rabbits and chickens. This immobility response should not be interpreted as loss of consciousness when the animal is, in fact, conscious. Distress vocalizations, fearful behavior, and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals. Therefore, for sensitive species, it is desirable that other animals not be present when individual animal euthanasia is performed.

HUMAN BEHAVIORAL CONSIDERATIONS

When animals must be euthanatized, either as individuals or in larger groups, moral and ethical concerns dictate that humane practices be observed. Human psychologic responses to euthanasia of animals need to be considered, with grief at the loss of a life as the most common reaction.[38] There are six circumstances under which the panel was most aware of the effects of animal euthanasia on people.

The first of these is the veterinary clinical setting where owners have to make decisions about whether and when to euthanatize. Although many owners rely heavily on their veterinarian's judgment, others may have misgivings about making their own decision. This

is particularly likely if an owner feels responsible for allowing an animal's medical or behavioral problem to go unattended so that euthanasia becomes necessary. When owners choose to be present during euthanasia, they should be prepared for what will happen. What drugs are being used and how the animal could respond should be discussed. Behaviors such as vocalization, muscle twitches, failure of the eyelids to close, urination, or defecation can be distressing. Counseling services for grieving owners are now available in some communities[39] and telephone counseling is available through some veterinary schools.[40,41] Owners are not the only people affected by euthanasia of animals. Veterinarians and their staffs may also become attached to patients they have known and treated for many years and may continue to struggle with the ethical implications of ending an animal's life.

The second is animal care and control facilities where unwanted, homeless, diseased, and injured animals must be euthanatized in large numbers. Distress may develop among personnel directly involved in performing euthanasia repeatedly. Emotional uneasiness, discomfort, or distress experienced by people involved with euthanasia of animals may be minimized. The person performing euthanasia must be technically proficient, use humane handling methods, understand the reasons for euthanasia, and be familiar with the method of euthanasia being employed (ie, what is going to happen to the animal). When the person is not knowledgeable about what to expect, he or she may mistakenly interpret any movement of animals as consciousness and a lack of movement as loss of consciousness. Methods that preclude movement of animals are more aesthetically acceptable to most technical staff even though lack of movement is not an adequate criterion for evaluating euthanasia techniques. Constant exposure to, or participation in, euthanasia procedures can cause a psychologic state characterized by a strong sense of work dissatisfaction or alienation, which may be expressed by absenteeism, belligerence, or careless and callous handling of animals.[42] This is one of the principal reasons for turnover of employees directly involved with repeated animal euthanasia. Management should be aware of potential personnel problems related to animal euthanasia and determine whether it is necessary to institute a program to prevent, decrease, or eliminate this problem. Specific coping strategies can make the task more tolerable. Some strategies include adequate training programs so that euthanasia is performed competently, peer support in the workplace, professional support as necessary, focusing on animals that are successfully adopted or returned to owners, devoting some work time to educational activities, and providing time off when workers feel stressed.

The third setting is the laboratory. Researchers, technicians, and students may become attached to animals that must be euthanatized.[43] The same considerations afforded pet owners or shelter employees should be provided to those working in laboratories.

The fourth situation is wildlife control. Wildlife biologists, wildlife managers, and wildlife health professionals are often responsible for euthanatizing animals that are injured, diseased, in excessive number, or that threaten property or human safety. Although relocation of some animals is appropriate and attempted, relocation is often only a temporary solution to a larger problem. People who must deal with these animals, especially under public pressure to save the animals rather than destroy them, can experience extreme distress and anxiety.

The fifth setting is livestock and poultry slaughter facilities. The large number of animals processed daily can take a heavy toll on employees physically and emotionally. Federal and state agricultural employees may also be involved in mass euthanasia of poultry and livestock in the face of disease outbreaks, bioterrorism, and natural disasters.

The last situation is public exposure. Because euthanasia of zoo animals, animals involved in roadside or racetrack accidents, stranded marine animals, nuisance or injured wildlife, and others can draw public attention, human attitudes and responses should be considered whenever animals are euthanatized. Natural disasters and foreign animal disease programs also present public challenges. These considerations, however, should not outweigh the primary responsibility of using the most rapid and painless euthanasia method possible under the circumstances.

MODES OF ACTION OF EUTHANATIZING AGENTS

Euthanatizing agents cause death by three basic mechanisms: (1) hypoxia, direct or indirect; (2) direct depression of neurons necessary for life function; and (3) physical disruption of brain activity and destruction of neurons necessary for life.

Agents that induce death by direct or indirect hypoxia can act at various sites and can cause loss of consciousness at different rates. For death to be painless and distress-free, loss of consciousness should pre-

cede loss of motor activity (muscle movement). Loss of motor activity, however, cannot be equated with loss of consciousness and absence of distress. Thus, agents that induce muscle paralysis without loss of consciousness are not acceptable as sole agents for euthanasia (eg, depolarizing and nondepolarizing muscle relaxants, strychnine, nicotine, and magnesium salts). With other techniques that induce hypoxia, some animals may have motor activity following loss of consciousness, but this is reflex activity and is not perceived by the animal.

A second group of euthanatizing agents depress nerve cells of the brain, inducing loss of consciousness followed by death. Some of these agents release inhibition of motor activity during the first stage of anesthesia, resulting in a so-called excitement or delirium phase, during which there may be vocalization and some muscle contraction. These responses do not appear to be purposeful. Death follows loss of consciousness, and is attributable to cardiac arrest and/or hypoxemia following direct depression of respiratory centers.

Physical disruption of brain activity, caused by concussion, direct destruction of the brain, or electrical depolarization of neurons, induces rapid loss of consciousness. Death occurs because of destruction of midbrain centers controlling cardiac and respiratory activity or as a result of adjunctive methods (eg, exsanguination) used to kill the animal. Exaggerated muscular activity can follow loss of consciousness and, although this may disturb some observers, the animal is not experiencing pain or distress.

INHALANT AGENTS

Any gas that is inhaled must reach a certain concentration in the alveoli before it can be effective; therefore, euthanasia with any of these agents takes some time. The suitability of a particular agent depends on whether an animal experiences distress between the time it begins to inhale the agent and the time it loses consciousness. Some agents may induce convulsions, but these generally follow loss of consciousness. Agents inducing convulsions prior to loss of consciousness are unacceptable for euthanasia.

Certain considerations are common to all inhalant agents. (1) In most cases, onset of loss of consciousness is more rapid, and euthanasia more humane, if the animal is rapidly exposed to a high concentration of the agent. (2) The equipment used to deliver and maintain this high concentration must be in good working order and in compliance with state and federal regulations. Leaky or faulty equipment may lead to slow, distressful death and be hazardous to other animals and to personnel. (3) Most of these agents are hazardous to personnel because of the risk of explosions (eg, ether), narcosis (eg, halothane), hypoxemia (eg, nitrogen and carbon monoxide), addiction (eg, nitrous oxide), or health effects resulting from chronic exposure (eg, nitrous oxide and carbon monoxide). (4) Alveolar concentrations rise slowly in an animal with decreased ventilation, making agitation more likely during induction. Other noninhalant methods of euthanasia should be considered for such animals. (5) Neonatal animals appear to be resistant to hypoxia, and because all inhalant agents ultimately cause hypoxia, neonatal animals take longer to die than adults. Glass et al,[44] reported that newborn dogs, rabbits, and guinea pigs survived a nitrogen atmosphere much longer than did adults. Dogs, at 1 week old, survived for 14 minutes compared with a 3-minute survival time after a few weeks of age. Guinea pigs survived for 4.5 minutes at 1 day old, compared with 3 minutes at 8 days or older. Rabbits survived for 13 minutes at 6 days old, 4 minutes at 14 days, and 1.5 minutes at 19 days and older. The panel recommended that inhalant agents not be used alone in animals less than 16 weeks old except to induce loss of consciousness, followed by the use of some other method to kill the animal. (6) Rapid gas flows can produce a noise that frightens animals. If high flows are required, the equipment should be designed to minimize noise. (7) Animals placed together in chambers should be of the same species, and, if needed, should be restrained so that they will not hurt themselves or others. Chambers should not be overloaded and need to be kept clean to minimize odors that might distress animals subsequently euthanatized. (8) Reptiles, amphibians, and diving birds and mammals have a great capacity for holding their breath and anaerobic metabolism. Therefore, induction of anesthesia and time to loss of consciousness when using inhalants may be greatly prolonged. Other techniques may be more appropriate for these species.

INHALANT ANESTHETICS

Inhalant anesthetics (eg, ether, halothane, methoxyflurane, isoflurane, sevoflurane, desflurane, and enflurane) have been used to euthanatize many species.[45] Halothane induces anesthesia rapidly and is the most effective inhalant anesthetic for euthanasia. Enflurane is less soluble in blood than halothane, but, because of its lower vapor pressure and lower potency, induction rates may be similar to those for halothane.

At deep anesthetic planes, animals may seizure. It is an effective agent for euthanasia, but the associated seizure activity may be disturbing to personnel. Isoflurane is less soluble than halothane, and it should induce anesthesia more rapidly. However, it has a slightly pungent odor and animals often hold their breath, delaying onset of loss of consciousness. Isoflurane also may require more drug to kill an animal, compared with halothane. Although isoflurane is acceptable as a euthanasia agent, halothane is preferred. Sevoflurane is less soluble than halothane and does not have an objectionable odor. It is less potent than isoflurane or halothane and has a lower vapor pressure. Anesthetic concentrations can be achieved and maintained rapidly. Desflurane is currently the least soluble potent inhalant anesthetic, but the vapor is quite pungent, which may slow induction. This drug is so volatile that it could displace oxygen (O_2) and induce hypoxemia during induction if supplemental O_2 is not provided. Methoxyflurane is highly soluble, and slow anesthetic induction with its use may be accompanied by agitation. It is a conditionally acceptable agent for euthanasia in rodents.[46] Ether has high solubility in blood and induces anesthesia slowly. It is irritating to the eyes and nose, poses serious risks associated with its flammability and explosiveness, and has been used to create a model for stress.[47-50]

With inhalant anesthetics, the animal can be placed in a closed receptacle containing cotton or gauze soaked with an appropriate amount of the anesthetic,[51] or the anesthetic can be introduced from a vaporizer. The latter method may be associated with a longer induction time. Vapors are inhaled until respiration ceases and death ensues. Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors. Also, sufficient air or O_2 must be provided during the induction period to prevent hypoxemia.[51] In the case of small rodents placed in a large container, there will be sufficient O_2 in the chamber to prevent hypoxemia. Larger species placed in small containers may need supplemental air or O_2.[51]

Nitrous oxide (N_2O) may be used with other inhalants to speed the onset of anesthesia, but alone it does not induce anesthesia in animals, even at 100% concentration. When used by itself, N_2O produces hypoxemia before respiratory or cardiac arrest. As a result, animals may become distressed prior to loss of consciousness.

Occupational exposure to inhalant anesthetics constitutes a human health hazard. Spontaneous abortion and congenital abnormalities have been associated with exposure of women to trace amounts of inhalation anesthetic agents during early stages of pregnancy.[52] Regarding human exposure to inhalant anesthetics, the concentrations of halothane, enflurane, and isoflurane should be less than 2 ppm, and less than 25 ppm for nitrous oxide.[52] There are no controlled studies proving that such concentrations of anesthetics are safe, but these concentrations were established because they were found to be attainable under hospital conditions. Effective procedures must be used to protect personnel from anesthetic vapors.

Advantages—(1) Inhalant anesthetics are particularly valuable for euthanasia of smaller animals (< 7 kg) or for animals in which venipuncture may be difficult. (2) Halothane, enflurane, isoflurane, sevoflurane, desflurane, methoxyflurane, and N_2O are nonflammable and nonexplosive under ordinary environmental conditions.

Disadvantages—(1) Animals may struggle and become anxious during induction of anesthesia because anesthetic vapors may be irritating and can induce excitement. (2) Ether is flammable and explosive. Explosions have occurred when animals, euthanatized with ether, were placed in an ordinary (not explosion proof) refrigerator or freezer and when bagged animals were placed in an incinerator. (3) Induction with methoxyflurane is unacceptably slow in some species. (4) Nitrous oxide will support combustion. (5) Personnel and animals can be injured by exposure to these agents. (6) There is a potential for human abuse of some of these drugs, especially N_2O.

Recommendations—In order of preference, halothane, enflurane, isoflurane, sevoflurane, methoxyflurane, and desflurane, with or without nitrous oxide, are acceptable for euthanasia of small animals (< 7 kg). Ether should only be used in carefully controlled situations in compliance with state and federal occupational health and safety regulations. It is conditionally acceptable. Nitrous oxide should not be used alone, pending further scientific studies on its suitability for animal euthanasia. Although acceptable, these agents are generally not used in larger animals because of their cost and difficulty of administration.

CARBON DIOXIDE

Room air contains 0.04% carbon dioxide (CO_2), which is heavier than air and nearly odorless. Inhalation of CO_2 at a concentration of 7.5% increases the pain threshold, and higher concentrations of CO_2 have a rapid anesthetic effect.[53-58]

Leake and Waters[56] reported the experimental use of CO_2 as an anesthetic agent for dogs. At concentrations of 30% to 40% CO_2 in O_2, anesthesia was induced within 1 to 2 minutes, usually without struggling, retching, or vomiting. For cats, inhalation of 60% CO_2 results in loss of consciousness within 45 seconds, and respiratory arrest within 5 minutes.[59] Signs of effective CO_2 anesthesia are those associated with deep surgical anesthesia, such as loss of withdrawal and palpebral reflexes.[60] Time to loss of consciousness is decreased by use of higher concentrations of CO_2 with an 80 to 100% concentration providing anesthesia in 12 to 33 seconds in rats and 70% CO_2 in O_2 inducing anesthesia in 40 to 50 seconds.[61,62] Time to loss of consciousness will be longer if the concentration is increased slowly rather than immersing the animal in the full concentration immediately.

Several investigators have suggested that inhalation of high concentrations of CO_2 may be distressing to animals,[63-66] because the gas dissolves in moisture on the nasal mucosa. The resulting product, carbonic acid, may stimulate nociceptors in the nasal mucosa. Some humans exposed to concentrations of around 50% CO_2 report that inhaling the gas is unpleasant and that higher concentrations are noxious.[67,68] A brief study of swine examined the aversive nature of CO_2 exposure69 and found that 90% CO_2 was aversive to pigs while 30% was not. For rats, exposure to increasing concentrations of CO_2 (33% achieved after 1 minute) in their home cage produced no evident stress as measured by behavior and ACTH, glucose, and corticosterone concentrations in serum.[70]

Carbon dioxide has been used to euthanatize groups of small laboratory animals, including mice, rats, guinea pigs, chickens, and rabbits,[5,71-76] and to render swine unconscious before humane slaughter. [22,63,64] The combination of 40% CO_2 and approximately 3% carbon monoxide (CO) has been used experimentally for euthanasia of dogs.[65] Carbon dioxide has been used in specially designed chambers to euthanatize individual cats[77,78] and other small laboratory animals.[51,72,79]

Studies of 1-day-old chickens have revealed that CO_2 is an effective euthanatizing agent. Inhalation of CO_2 caused little distress to the birds, suppressed nervous activity, and induced death within 5 minutes.[73] Because respiration begins during embryonic development, the unhatched chicken's environment may normally have a CO_2 concentration as high as 14%. Thus, CO_2 concentrations for euthanasia of newly hatched chickens and neonates of other species should be especially high. A CO_2 concentration of 60% to 70% with a 5-minute exposure time appears to be optimal.[73]

In studies of mink, high concentrations of CO_2 would kill them quickly, but a 70% CO_2 concentration induced loss of consciousness without killing them.[80] Some burrowing animals, such as rabbits of the species *Oryctolagus,* also have prolonged survival times when exposed to CO_2.[81] Some burrowing and diving animals have physiologic mechanisms for coping with hypercapnia. Therefore, it is necessary to have a sufficient concentration of CO_2 to kill the animal by hypoxemia following induction of anesthesia with CO_2.

Advantages—(1) The rapid depressant, analgesic, and anesthetic effects of CO_2 are well established. (2) Carbon dioxide is readily available and can be purchased in compressed gas cylinders. (3) Carbon dioxide is inexpensive, nonflammable, nonexplosive, and poses minimal hazard to personnel when used with properly designed equipment. (4) Carbon dioxide does not result in accumulation of tissue residues in food-producing animals. (5) Carbon dioxide euthanasia does not distort murine cholinergic markers[82] or corticosterone concentrations.83

Disadvantages—(1) Because CO_2 is heavier than air, incomplete filling of a chamber may permit animals to climb or raise their heads above the higher concentrations and avoid exposure. (2) Some species, such as fish and burrowing and diving mammals, may have extraordinary tolerance for CO_2. (3) Reptiles and amphibians may breathe too slowly for the use of CO_2. (4) Euthanasia by exposure to CO_2 may take longer than euthanasia by other means.[61] (5) Induction of loss of consciousness at lower concentrations (< 80%) may produce pulmonary and upper respiratory tract lesions.[67,84] (6) High concentrations of CO_2 may be distressful to some animals.

Recommendations—Carbon dioxide is acceptable for euthanasia in appropriate species (**Appendices 1 and 2).** Compressed CO_2 gas in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (eg, antacids) is unacceptable. Species should be separated and chambers should not be overcrowded. With an animal in the chamber, an optimal flow rate should displace at least 20% of the chamber volume per minute.[85] Loss of consciousness may be induced more rapidly by exposing animals to a CO_2 concentration of 70% or more by prefilling the chamber for species in which this has not been shown to cause distress. Gas flow should be

maintained for at least 1 minute after apparent clinical death.[86] It is important to verify that an animal is dead before removing it from the chamber. If an animal is not dead, CO_2 narcosis must be followed with another method of euthanasia. Adding O_2 to the CO_2 may or may not preclude signs of distress.67,87 Additional O_2 will, however, prolong time to death and may complicate determination of consciousness. There appears to be no advantage to combining O_2 with carbon dioxide for euthanasia.[87]

NITROGEN, ARGON

Nitrogen (N_2) and argon (Ar) are colorless, odorless gases that are inert, nonflammable, and nonexplosive. Nitrogen comprises 78% of atmospheric air, whereas Ar comprises less than 1%.

Euthanasia is induced by placing the animal in a closed container that has been prefilled with N_2 or Ar or into which the gas is then rapidly introduced. Nitrogen/Ar displaces O_2, thus inducing death by hypoxemia.

In studies by Herin et al,[88] dogs became unconscious within 76 seconds when a N_2 concentration of 98.5% was achieved in 45 to 60 seconds. The electroencephalogram (EEG) became isoelectric (flat) in a mean time of 80 seconds, and arterial blood pressure was undetectable at 204 seconds. Although all dogs hyperventilated prior to loss of consciousness, the investigators concluded that this method induced death without pain. Following loss of consciousness, vocalization, gasping, convulsions, and muscular tremors developed in some dogs. At the end of a 5-minute exposure period, all dogs were dead.[88] These findings were similar to those for rabbits[89] and mink.[80,90]

With N_2 flowing at a rate of 39% of chamber volume per minute, rats collapsed in approximately 3 minutes and stopped breathing in 5 to 6 minutes. Regardless of flow rate, signs of panic and distress were evident before the rats collapsed and died.[85] Insensitivity to pain under such circumstances is questionable.[91]

Tranquilization with acepromazine, in conjunction with N_2 euthanasia of dogs, was investigated by Quine et al.[92] Using ECG and EEG recordings, they found these dogs had much longer survival times than dogs not given acepromazine before administration of N_2. In one dog, ECG activity continued for 51 minutes. Quine also addressed distress associated with exposure to N_2 by removing cats and dogs from the chamber following loss of consciousness and allowing them to recover. When these animals were put back into the chamber, they did not appear afraid or apprehensive.

Investigations into the aversiveness of Ar to swine and poultry have revealed that these animals will tolerate breathing 90% Ar with 2% O_2.[69,71] Swine voluntarily entered a chamber containing this mixture, for a food reward, and only withdrew from the chamber as they became ataxic. They reentered the chamber immediately to continue eating. Poultry also entered a chamber containing this mixture for a food reward and continued eating until they collapsed.[71] When Ar was used to euthanatize chickens, exposure to a chamber prefilled with Ar, with an O_2 concentration of < 2%, led to EEG changes and collapse in 9 to 12 seconds. Birds removed from the chamber at 15 to 17 seconds failed to respond to comb pinching. Continued exposure led to convulsions at 20 to 24 seconds. Somatosensory-evoked potentials were lost at 24 to 34 seconds, and the EEG became isoelectric at 57 to 66 seconds. Convulsion onset was after loss of consciousness (collapse and loss of response to comb pinch), so this would appear to be a humane method of euthanasia for chickens.[93] Despite the availability of some information, there is still much about the use of N_2/Ar that needs to be investigated.

Advantages—(1) Nitrogen and Ar are readily available as compressed gases. (2) Hazards to personnel are minimal.

Disadvantages—(1) Loss of consciousness is preceded by hypoxemia and ventilatory stimulation, which may be distressing to the animal. (2) Reestablishing a low concentration of O_2 (ie, 6% or greater) in the chamber before death will allow immediate recovery.[69]

Recommendations—Nitrogen and Ar can be distressful to some species (eg, rats).[85] Therefore, this technique is conditionally acceptable only if O_2 concentrations <2% are achieved rapidly, and animals are heavily sedated or anesthetized. With heavy sedation or anesthesia, it should be recognized that death may be delayed. Although N_2 and Ar are effective, other methods of euthanasia are preferable.

CARBON MONOXIDE

Carbon monoxide (CO) is a colorless, odorless gas that is nonflammable and nonexplosive unless concentrations exceed 10%. It combines with hemoglobin to form carboxyhemoglobin and blocks uptake of O_2 by erythrocytes, leading to fatal hypoxemia.

In the past, mass euthanasia has been accomplished by use of 3 methods for generating CO: (1) chemical interaction of sodium formate and sulfuric acid, (2) exhaust fumes from idling gasoline internal

combustion engines, and (3) commercially compressed CO in cylinders. The first 2 techniques are associated with problems such as production of other gases, achieving inadequate concentrations of carbon monoxide, inadequate cooling of the gas, and maintenance of equipment. Therefore, the only acceptable source is compressed CO in cylinders.

In a study by Ramsey and Eilmann,[94] 8% CO caused guinea pigs to collapse in 40 seconds to 2 minutes, and death occurred within 6 minutes. Carbon monoxide has been used to euthanatize mink[80,90] and chinchillas. These animals collapsed in 1 minute, breathing ceased in 2 minutes, and the heart stopped beating in 5 to 7 minutes.

In a study evaluating the physiologic and behavioral characteristics of dogs exposed to 6% CO in air, Chalifoux and Dallaire[95] could not determine the precise time of loss of consciousness. Electroencephalographic recordings revealed 20 to 25 seconds of abnormal cortical function prior to loss of consciousness. It was during this period that the dogs became agitated and vocalized. It is not known whether animals experience distress; however, humans in this phase reportedly are not distressed.[96] Subsequent studies have revealed that tranquilization with acepromazine significantly decreases behavioral and physiologic responses of dogs euthanatized with CO.[97]

In a comparative study, CO from gasoline engine exhaust and 70% CO_2 plus 30% O_2 were used to euthanatize cats. Euthanasia was divided into 3 phases. Phase I was the time from initial contact to onset of clinical signs (eg, yawning, staggering, or trembling). Phase II extended from the end of phase I until recumbency, and phase III from the end of phase II until death.[54] The study revealed that signs of agitation before loss of consciousness were greatest with CO_2 plus O_2. Convulsions occurred during phases II and III with both methods. However, when the euthanasia chamber was prefilled with CO (ie, exhaust fumes), convulsions did not occur in phase III. Time to complete immobilization was greater with CO_2 plus O_2 (approximately 90 seconds) than with CO alone (approximately 56 seconds).[54] In neonatal pigs, excitation was more likely to precede loss of consciousness if the pigs were exposed to a rapid rise in CO concentration. This agitation was reduced at lower flow rates, or when CO was combined with nitrogen.[98]

In people, the most common symptoms of early CO toxicosis are headache, dizziness, and weakness. As concentrations of carboxyhemoglobin increase, these signs may be followed by decreased visual acuity, tinnitus, nausea, progressive depression, confusion, and collapse.99 Because CO stimulates motor centers in the brain, loss of consciousness may be accompanied by convulsions and muscular spasms.

Carbon monoxide is a cumulative poison.[96] Distinct signs of CO toxicosis are not evident until the CO concentration is 0.05% in air, and acute signs do not develop until the CO concentration is approximately 0.2% in air. In humans, exposure to 0.32% CO and 0.45% CO for one hour will induce loss of consciousness and death, respectively.[100] Carbon monoxide is extremely hazardous for personnel because it is highly toxic and difficult to detect. Chronic exposure to low concentrations of carbon monoxide may be a health hazard, especially with regard to cardiovascular disease and teratogenic effects.[101-103] An efficient exhaust or ventilatory system is essential to prevent accidental exposure of humans.

Advantages—(1) Carbon monoxide induces loss of consciousness without pain and with minimal discernible discomfort. (2) Hypoxemia induced by CO is insidious, so that the animal appears to be unaware. (3) Death occurs rapidly if concentrations of 4 to 6% are used.

Disadvantages—(1) Safeguards must be taken to prevent exposure of personnel. (2) Any electrical equipment exposed to CO (eg, lights and fans) must be explosion proof.

Recommendations—Carbon monoxide used for individual animal or mass euthanasia is acceptable for dogs, cats, and other small mammals, provided that commercially compressed CO is used and the following precautions are taken: (1) personnel using CO must be instructed thoroughly in its use and must understand its hazards and limitations; (2) the CO chamber must be of the highest quality construction and should allow for separation of individual animals; (3) the CO source and chamber must be located in a well-ventilated environment, preferably out of doors; (4) the chamber must be well lit and have view ports that allow personnel direct observation of animals; (5) the CO flow rate should be adequate to rapidly achieve a uniform CO concentration of at least 6% after animals are placed in the chamber, although some species (eg, neonatal pigs) are less likely to become agitated with a gradual rise in CO concentration;[98] and (6) if the chamber is inside a room, CO monitors must be placed in the room to warn personnel of hazardous concentrations. It is essential that CO use be in compliance with state and federal occupational health and safety regulations.

NONINHALANT PHARMACEUTICAL AGENTS

The use of injectable euthanasia agents is the most rapid and reliable method of performing euthanasia. It is the most desirable method when it can be performed without causing fear or distress in the animal. When the restraint necessary for giving an animal an intravenous injection would impart added distress to the animal or pose undue risk to the operator, sedation, anesthesia, or an acceptable alternate route of administration should be employed. Aggressive, fearful, wild, or feral animals should be sedated or given a nonparalytic immobilizing agent prior to intravenous administration of the euthanasia agent.

When intravenous administration is considered impractical or impossible, intraperitoneal administration of a nonirritating euthanasia agent is acceptable, provided the drug does not contain neuromuscular blocking agents. Intracardiac injection is acceptable only when performed on heavily sedated, anesthetized, or comatose animals. It is not considered acceptable in awake animals, owing to the difficulty and unpredictability of performing the injection accurately. Intramuscular, subcutaneous, intrathoracic, intrapulmonary, intrahepatic, intrarenal, intrasplenic, intrathecal, and other nonvascular injections are not acceptable methods of administering injectable euthanasia agents.

When injectable euthanasia agents are administered into the peritoneal cavity, animals may be slow to pass through stages I and II of anesthesia. Accordingly, they should be placed in small cages in a quiet area to minimize excitement and trauma.

BARBITURIC ACID DERIVATIVES

Barbiturates depress the central nervous system in descending order, beginning with the cerebral cortex, with loss of consciousness progressing to anesthesia. With an overdose, deep anesthesia progresses to apnea, owing to depression of the respiratory center, which is followed by cardiac arrest.

All barbituric acid derivatives used for anesthesia are acceptable for euthanasia when administered intravenously. There is a rapid onset of action, and loss of consciousness induced by barbiturates results in minimal or transient pain associated with venipuncture. Desirable barbiturates are those that are potent, long-acting, stable in solution, and inexpensive. Sodium pentobarbital best fits these criteria and is most widely used, although others such as secobarbital are also acceptable.

Advantages—(1) A primary advantage of barbiturates is speed of action. This effect depends on the dose, concentration, route, and rate of the injection. (2) Barbiturates induce euthanasia smoothly, with minimal discomfort to the animal. (3) Barbiturates are less expensive than many other euthanasia agents.

Disadvantages—(1) Intravenous injection is necessary for best results and requires trained personnel. (2) Each animal must be restrained. (3) Current federal drug regulations require strict accounting for barbiturates and these must be used under the supervision of personnel registered with the US Drug Enforcement Administration (DEA). (4) An aesthetically objectionable terminal gasp may occur in unconscious animals. (5) These drugs tend to persist in the carcass and may cause sedation or even death of animals that consume the body.

Recommendations—The advantages of using barbiturates for euthanasia in small animals far outweigh the disadvantages. Intravenous injection of a barbituric acid derivative is the preferred method for euthanasia of dogs, cats, other small animals, and horses. Intraperitoneal injection may be used in situations when an intravenous injection would be distressful or even dangerous. Intracardiac injection must only be used if the animal is heavily sedated, unconscious, or anesthetized.

PENTOBARBITAL COMBINATIONS

Several euthanasia products are formulated to include a barbituric acid derivative (usually sodium pentobarbital), with added local anesthetic agents or agents that metabolize to pentobarbital. Although some of these additives are slowly cardiotoxic, this pharmacologic effect is inconsequential. These combination products are listed by the DEA as Schedule III drugs, making them somewhat simpler to obtain, store, and administer than Schedule II drugs such as sodium pentobarbital. The pharmacologic properties and recommended use of combination products that combine sodium pentobarbital with lidocaine or phenytoin are interchangeable with those of pure barbituric acid derivatives.

A combination of pentobarbital with a neuromuscular blocking agent is not an acceptable euthanasia agent.

CHLORAL HYDRATE

Chloral hydrate depresses the cerebrum slowly; therefore, restraint may be a problem for some animals. Death is caused by hypoxemia resulting from progressive depression of the respiratory center, and may be

preceded by gasping, muscle spasms, and vocalization.

Recommendations—Chloral hydrate is conditionally acceptable for euthanasia of large animals only when administered intravenously, and only after sedation to decrease the aforementioned undesirable side effects. Chloral hydrate is not acceptable for dogs, cats, and other small animals because the side effects may be severe, reactions can be aesthetically objectionable, and other products are better choices.

T-61

T-61 is an injectable, nonbarbiturate, non-narcotic mixture of 3 drugs used for euthanasia. These drugs provide a combination of general anesthetic, curariform, and local anesthetic actions. T-61 has been withdrawn from the market and is no longer manufactured or commercially available in the United States. It is available in Canada and other countries. T-61 should be used only intravenously and at carefully monitored rates of injection, because there is some question as to the differential absorption and onset of action of the active ingredients when administered by other routes.[1]

TRICAINE METHANE SULFONATE (MS 222, TMS)

MS 222 is commercially available as tricaine methane sulfonate (TMS), which can be used for the euthanasia of amphibians and fish. Tricaine is a benzoic acid derivative and, in water of low alkalinity (< 50 mg/L as CaCO3); the solution should be buffered with sodium bicarbonate.104 A 10 g/L stock solution can be made, and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution. The stock solution should be stored in a dark brown bottle, and refrigerated or frozen if possible. The solution should be replaced monthly and any time a brown color is observed.105 For euthanasia, a concentration ≥ 250 mg/L is recommended and fish should be left in this solution for at least 10 minutes following cessation of opercular movement.104 In the United States, there is a 21-day withdrawal time for MS 222; therefore, it is not appropriate for euthanasia of animals intended for food.

POTASSIUM CHLORIDE IN CONJUNCTION WITH PRIOR GENERAL ANESTHESIA

Although unacceptable and condemned when used in unanaesthetized animals, the use of a supersaturated solution of potassium chloride injected intravenously or intracardially in an animal under general anesthesia is an acceptable method to produce cardiac arrest and death. The potassium ion is cardiotoxic, and rapid intravenous or intracardiac administration of 1 to 2 mmol/kg of body weight will cause cardiac arrest. This is a preferred injectable technique for euthanasia of livestock or wildlife species to reduce the risk of toxicosis for predators or scavengers in situations where carcasses of euthanatized animals may be consumed.[106,107]

Advantages—(1) Potassium chloride is not a controlled substance. It is easily acquired, transported, and mixed in the field. (2) Potassium chloride, when used with appropriate methods to render an animal unconscious, results in a carcass that is potentially less toxic for scavengers and predators in cases where carcass disposal is impossible or impractical.

Disadvantage—Rippling of muscle tissue and clonic spasms may occur on or shortly after injection.

Recommendations—It is of utmost importance that personnel performing this technique are trained and knowledgeable in anesthetic techniques, and are competent in assessing anesthetic depth appropriate for administration of potassium chloride intravenously. Administration of potassium chloride intravenously requires animals to be in a surgical plane of anesthesia characterized by loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli. Saturated potassium chloride solutions are effective in causing cardiac arrest following rapid intracardiac or intravenous injection. Residual tissue concentrations of general anesthetics after anesthetic induction have not been documented. Whereas no scavenger toxicoses have been reported with potassium chloride in combination with a general anesthetic, proper carcass disposal should always be attempted to prevent possible toxicosis by consumption of a carcass contaminated with general anesthetics.

UNACCEPTABLE INJECTABLE AGENTS

When used alone, the injectable agents listed in **Appendix 4** (strychnine, nicotine, caffeine, magnesium sulfate, potassium chloride, cleaning agents, solvents, disinfectants and other toxins or salts, and all neuromuscular blocking agents) are unacceptable and are absolutely condemned for use as euthanasia agents.

PHYSICAL METHODS

Physical methods of euthanasia include captive bolt, gunshot, cervical dislocation, decapitation, electrocution, microwave irradiation, kill traps, thoracic compression, exsanguination, maceration, stunning,

and pithing. When properly used by skilled personnel with well-maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of euthanasia. Exsanguination, stunning, and pithing are not recommended as a sole means of euthanasia, but should be considered adjuncts to other agents or methods.

Some consider physical methods of euthanasia aesthetically displeasing. There are occasions, however, when what is perceived as aesthetic and what is most humane are in conflict. Physical methods may be the most appropriate method for euthanasia and rapid relief of pain and suffering in certain situations. Personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique performed. That person must also be sensitive to the aesthetic implications of the method and inform onlookers about what they should expect when possible.

Since most physical methods involve trauma, there is inherent risk for animals and humans. Extreme care and caution should be used. Skill and experience of personnel is essential. If the method is not performed correctly, animals and personnel may be injured. Inexperienced persons should be trained by experienced persons and should practice on carcasses or anesthetized animals to be euthanatized until they are proficient in performing the method properly and humanely. When done appropriately, the panel considered most physical methods conditionally acceptable for euthanasia.

PENETRATING CAPTIVE BOLT

A penetrating captive bolt is used for euthanasia of ruminants, horses, swine, laboratory rabbits, and dogs.[108] Its mode of action is concussion and trauma to the cerebral hemisphere and brainstem.[109,110] Captive bolt guns are powered by gunpowder or compressed air and must provide sufficient energy to penetrate the skull of the species on which they are being used.[109] Adequate restraint is important to ensure proper placement of the captive bolt. A cerebral hemisphere and the brainstem must be sufficiently disrupted by the projectile to induce sudden loss of consciousness and subsequent death. Accurate placement of captive bolts for various species has been described.[109-112] A multiple projectile has been suggested as a more effective technique, especially for large cattle.[109]

A nonpenetrating captive bolt only stuns animals and should not be used as a sole means of euthanasia (see “Stunning” under “Adjunctive Methods”).

Advantage—The penetrating captive bolt is an effective method of euthanasia for use in slaughter-houses, in research facilities, and on the farm when use of drugs is inappropriate.

Disadvantages—(1) It is aesthetically displeasing. (2) Death may not occur if equipment is not maintained and used properly.

Recommendations—Use of the penetrating captive bolt is an acceptable and practical method of euthanasia for horses, ruminants, and swine. It is conditionally acceptable in other appropriate species. The non-penetrating captive bolt must not be used as a sole method of euthanasia.

EUTHANASIA BY A BLOW TO THE HEAD

Euthanasia by a blow to the head must be evaluated in terms of the anatomic features of the species on which it is to be performed. A blow to the head can be a humane method of euthanasia for neonatal animals with thin craniums, such as young pigs, if a single sharp blow delivered to the central skull bones with sufficient force can produce immediate depression of the central nervous system and destruction of brain tissue. When properly performed, loss of consciousness is rapid. The anatomic features of neonatal calves, however, make a blow to the head in this species unacceptable. Personnel performing euthanasia by use of a blow to the head must be properly trained and monitored for proficiency with this method of euthanasia, and they must be aware of its aesthetic implications.

GUNSHOT

A properly placed gunshot can cause immediate insensibility and humane death. In some circumstances, a gunshot may be the only practical method of euthanasia. Shooting should only be performed by highly skilled personnel trained in the use of firearms and only in jurisdictions that allow for legal firearm use. Personnel, public, and nearby animal safety should be considered. The procedure should be performed outdoors and away from public access.

For use of a gunshot to the head as a method of euthanasia in captive animals, the firearm should be aimed so that the projectile enters the brain, causing instant loss of consciousness.[51,112-114] This must take into account differences in brain position and skull conformation between species, as well as the energy requirement for skull bone and sinus penetration.[109,115] Accurate targeting for a gunshot to the head in various species has been described.[114,116-119] For wildlife and

other freely roaming animals, the preferred target area should be the head. The appropriate firearm should be selected for the situation, with the goal being penetration and destruction of brain tissue without emergence from the contralateral side of the head.[120] A gunshot to the heart or neck does not immediately render animals unconscious and thus is not considered to meet the panel's definition of euthanasia.[121]

Advantages—(1) Loss of consciousness is instantaneous if the projectile destroys most of the brain. (2) Given the need to minimize stress induced by handling and human contact, gunshot may at times be the most practical and logical method of euthanasia of wild or free-ranging species.

Disadvantages—(1) Gunshot may be dangerous to personnel. (2) It is aesthetically unpleasant. (3) Under field conditions, it may be difficult to hit the vital target area. (4) Brain tissue may not be able to be examined for evidence of rabies infection or chronic wasting disease when the head is targeted.

Recommendations—When other methods cannot be used, an accurately delivered gunshot is a conditionally acceptable method of euthanasia.114,122-125 When an animal can be appropriately restrained, the penetrating captive bolt is preferred to a gunshot. Prior to shooting, animals accustomed to the presence of humans should be treated in a calm and reassuring manner to minimize anxiety. In the case of wild animals, gunshots should be delivered with the least amount of prior human contact necessary. Gunshot should not be used for routine euthanasia of animals in animal control situations, such as municipal pounds or shelters.

CERVICAL DISLOCATION

Cervical dislocation is a technique that has been used for many years and, when performed by well-trained individuals, appears to be humane. However, there are few scientific studies to confirm this observation. This technique is used to euthanatize poultry, other small birds, mice, and immature rats and rabbits. For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. For immature rabbits, the head is held in one hand and the hind limbs in the other. The animal is stretched and the neck is hyperextended and dorsally twisted to separate the first cervical vertebra from the skull.[72, 111] For poultry, cervical dislocation by stretching is a common method for mass euthanasia, but loss of consciousness may not be instantaneous.[134]

Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation, [127] and unlike decapitation, rapid exsanguination does not contribute to loss of consciousness.[128, 129]

Advantages—(1) Cervical dislocation is a technique that may induce rapid loss of consciousness.[84, 127] (2) It does not chemically contaminate tissue. (3) It is rapidly accomplished.

Disadvantages—(1) Cervical dislocation may be aesthetically displeasing to personnel. (2) Cervical dislocation requires mastering technical skills to ensure loss of consciousness is rapidly induced. (3) Its use is limited to poultry, other small birds, mice, and immature rats and rabbits.

Recommendations—Manual cervical dislocation is a humane technique for euthanasia of poultry, other small birds, mice, rats weighing < 200 g, and rabbits weighing < 1 kg when performed by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be sedated or anesthetized prior to cervical dislocation. The need for technical competency is greater in heavy rats and rabbits, in which the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult.[130] In research settings, this technique should be used only when scientifically justified by the user and approved by the Institutional Animal Care and Use Committee.

Those responsible for the use of this technique must ensure that personnel performing cervical dislocation techniques have been properly trained and consistently apply it humanely and effectively.

DECAPITATION

Decapitation can be used to euthanatize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study.[131]

Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation,[132] more recent studies and reports indicate that this activity does not infer the ability to perceive pain, and in fact conclude that loss of consciousness develops rapidly.[127-129]

Guillotines that are designed to accomplish decapitation in adult rodents and small rabbits in a uniformly

instantaneous manner are commercially available.

Guillotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose.

Advantages—(1) Decapitation is a technique that appears to induce rapid loss of consciousness.[127-129] (2) It does not chemically contaminate tissues. (3) It is rapidly accomplished.

Disadvantages—(1) Handling and restraint required to perform this technique may be distressful to animals.[83] (2) The interpretation of the presence of electrical activity in the brain following decapitation has created controversy and its importance may still be open to debate.[127-129,132] (3) Personnel performing this technique should recognize the inherent danger of the guillotine and take adequate precautions to prevent personal injury. (4) Decapitation may be aesthetically displeasing to personnel performing or observing the technique.

Recommendations—This technique is conditionally acceptable if performed correctly, and it should be used in research settings when its use is required by the experimental design and approved by the Institutional Animal Care and Use Committee. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine. Decapitation of amphibians, fish, and reptiles is addressed elsewhere in these guidelines.

Those responsible for the use of this technique must ensure that personnel who perform decapitation techniques have been properly trained to do so.

ELECTROCUTION

Electrocution, using alternating current, has been used as a method of euthanasia for species such as dogs, cattle, sheep, swine, foxes, and mink.[113,133-138] Electrocution induces death by cardiac fibrillation, which causes cerebral hypoxia.[135,137,139] However, animals do not lose consciousness for 10 to 30 seconds or more after onset of cardiac fibrillation. It is imperative that animals be unconscious before being electrocuted. This can be accomplished by any acceptable means, including electrical stunning.[25] Although an effective, 1-step stunning and electrocution method has been described for use in sheep and hogs, euthanasia by electrocution in most species remains a 2-step procedure.[25, 63,140]

Advantages—(1) Electrocution is humane if the animal is first rendered unconscious. (2) It does not chemically contaminate tissues. (3) It is economical.

Disadvantages—(1) Electrocution may be hazardous to personnel. (2) When conventional single-animal probes are used, it may not be a useful method for mass euthanasia because so much time is required per animal. (3) It is not a useful method for dangerous, intractable animals. (4) It is aesthetically objectionable because of violent extension and stiffening of the limbs, head, and neck. (5) It may not result in death in small animals (< 5 kg) because ventricular fibrillation and circulatory collapse do not always persist after cessation of current flow.

Recommendations—Euthanasia by electrocution requires special skills and equipment that will ensure passage of sufficient current through the brain to induce loss of consciousness and cardiac fibrillation in the 1-step method for sheep and hogs, or cardiac fibrillation in the unconscious animal when the 2-step procedure is used. Although the method is conditionally acceptable if the aforementioned requirements are met, its disadvantages far outweigh its advantages in most applications. Techniques that apply electric current from head to tail, head to foot, or head to moistened metal plates on which the animal is standing are unacceptable.

MICROWAVE IRRADIATION

Heating by microwave irradiation is used primarily by neurobiologists to fix brain metabolites *in vivo* while maintaining the anatomic integrity of the brain.[141] Microwave instruments have been specifically designed for use in euthanasia of laboratory mice and rats. The instruments differ in design from kitchen units and may vary in maximal power output from 1.3 to 10 kw. All units direct their microwave energy to the head of the animal. The power required to rapidly halt brain enzyme activity depends on the efficiency of the unit, the ability to tune the resonant cavity and the size of the rodent head.[142] There is considerable variation among instruments in the time required for loss of consciousness and euthanasia. A 10 kw, 2,450 MHz instrument operated at a power of 9 kw will increase the brain temperature of 18 to 28 g mice to 79 C in 330 ms, and the brain temperature of 250 to 420 g rats to 94 C in 800 ms.[143]

Advantages—(1) Loss of consciousness is achieved in less than 100 ms, and death in less than 1 second. (2) This is the most effective method to fix brain tissue *in vivo* for subsequent assay of enzymatically

labile chemicals.

Disadvantages—(1) Instruments are expensive. (2) Only animals the size of mice and rats can be euthanatized with commercial instruments that are currently available.

Recommendations—Microwave irradiation is a humane method for euthanatizing small laboratory rodents if instruments that induce rapid loss of consciousness are used. Only instruments that are designed for this use and have appropriate power and microwave distribution can be used. Microwave ovens designed for domestic and institutional kitchens are absolutely unacceptable for euthanasia.

THORACIC (CARDIOPULMONARY, CARDIAC) COMPRESSION

Thoracic (cardiopulmonary, cardiac) compression is used to euthanatize small- to medium-sized free-ranging birds when alternate techniques described in these guidelines are not practical.[144]

Advantages—(1) This technique is rapid. (2) It is apparently painless. (3) It maximizes carcass use for analytical/contaminant studies.

Disadvantages—(1) It may be considered aesthetically unpleasant by onlookers. (2) The degree of distress is unknown.

Recommendations—Thoracic (cardiopulmonary, cardiac) compression is a physical technique for avian euthanasia that has applicability in the field when other methods cannot be used. It is accomplished by bringing the thumb and forefinger of one hand under the bird's wing from the posterior and placing them against the ribs.[144] The forefinger of the other hand is placed against the ventral edge of the sternum, just below the furculum. All fingers are brought together forcefully and held under pressure to stop the heart and lungs. Loss of consciousness and death develop quickly. Proper training is needed in the use of this technique to avoid trauma to the bird. Cardiopulmonary compression is not appropriate for laboratory settings, for large or diving birds,[144] or for other species.

KILL TRAPS

Mechanical kill traps are used for the collection and killing of small, free-ranging mammals for commercial purposes (fur, skin, or meat), scientific purposes, to stop property damage, and to protect human safety. Their use remains controversial, and the panel recognized that kill traps do not always render a rapid or stress-free death consistent with criteria for euthanasia found elsewhere in this document. For this reason, use of live traps followed by other methods of euthanasia is preferred. There are a few situations when that is not possible or when it may actually be more stressful to the animals or dangerous to humans to use live traps. Although newer technologies are improving kill trap performance in achieving loss of consciousness quickly, individual testing is recommended to be sure the trap is working properly.[145] If kill traps must be used, the most humane available must be chosen,[146-148] as evaluated by use of International Organization for Standardization (ISO) testing procedures,[149] or by the methods of Gilbert,[150] Proulx et al,[151,152] or Hiltz and Roy.[153]

To reach the required level of efficiency, traps may need to be modified from manufacturers' production standards. In addition, as specified in scientific studies, trap placement (ground versus tree sets), bait type, set location, selectivity apparatus, body placement modifying devices (eg, sidewings, cones), trigger sensitivity, and trigger type, size, and conformation are essential considerations that could affect a kill trap's ability to reach these standards.

Several kill traps, modifications, and set specifics have been scientifically evaluated and found to meet the afore referenced standards for various species.[151, 152,154-167]

Advantage—Free-ranging small mammals may be killed with minimal distress associated with handling and human contact.

Disadvantages—(1) Traps may not afford death within acceptable time periods. (2) Selectivity and efficiency is dependent on the skill and proficiency of the operator.

Recommendations—Kill traps do not always meet the panel's criteria for euthanasia. At the same time, it is recognized that they can be practical and effective for scientific animal collection when used in a manner that ensures selectivity, a swift kill, no damage to body parts needed for field research, and minimal potential for injury of nontarget species.[168,169] Traps need to be checked at least once daily. In those instances when an animal is wounded or captured but not dead, the animal must be killed quickly and humanely. Kill traps should be used only when other acceptable techniques are impossible or have failed. Traps for nocturnal species should not be activated during the day to avoid capture of diurnal species.[168] Trap manufacturers should strive to meet their responsibility of minimizing pain and suffering in target species.

MACERATION

Maceration, via use of a specially designed mechanical apparatus having rotating blades or projections, causes immediate fragmentation and death of day-old poultry and embryonated eggs. A review[217] of the use of commercially available macerators for euthanasia of chicks, poults, and pipped eggs indicates that death by maceration in day-old poultry occurs immediately with minimal pain and distress. Maceration is an alternative to the use of carbon dioxide for euthanasia of day-old poultry. Maceration is believed to be equivalent to cervical dislocation and cranial compression as to time element, and is considered to be an acceptable means of euthanasia for newly hatched poultry by the Federation of Animal Science Societies,[220] Agriculture Canada,[221] World Organization for Animal Health (OIE),[222] and European Union.[223]

Advantages—(1) Death is almost instantaneous. (2) The method is safe for workers. (3) Large numbers of animals can be killed quickly.

Disadvantages—(1) Special equipment is required. (2) Macerated tissues may present biosecurity risks.

Recommendations—Maceration requires special equipment that must be kept in excellent working order. Chicks must be delivered to the macerator in a way and at a rate that prevents a backlog of chicks at the point of entry into the macerator and without causing injury, suffocation, or avoidable distress to the chicks before maceration.

ADJUNCTIVE METHODS

Stunning and pithing, when properly done, induce loss of consciousness but do not ensure death. Therefore, these methods must be used only in conjunction with other procedures,[123] such as pharmacologic agents, exsanguination, or decapitation to euthanatize the animal.

Exsanguination

Exsanguination can be used to ensure death subsequent to stunning, or in otherwise unconscious animals. Because anxiety is associated with extreme hypovolemia, exsanguination must not be used as a sole means of euthanasia.[170] Animals may be exsanguinated to obtain blood products, but only when they are sedated, stunned, or anesthetized.[171]

Stunning

Animals may be stunned by a blow to the head, by use of a nonpenetrating captive bolt, or by use of electric current. Stunning must be followed immediately by a method that ensures death. With stunning, evaluating loss of consciousness is difficult, but it is usually associated with a loss of the menace or blink response, pupillary dilatation, and a loss of coordinated movements. Specific changes in the electroencephalogram and a loss of visually evoked responses are also thought to indicate loss of consciousness.[60, 172]

Blow to the head—Stunning by a blow to the head is used primarily in small laboratory animals with thin craniums.[9, 173-175] A single sharp blow must be delivered to the central skull bones with sufficient force to produce immediate depression of the central nervous system. When properly done, consciousness is lost rapidly.

Nonpenetrating captive bolt—A nonpenetrating captive bolt may be used to induce loss of consciousness in ruminants, horses, and swine. Signs of effective stunning by captive bolt are immediate collapse and a several second period of tetanic spasm, followed by slow hind limb movements of increasing frequency.[60,176] Other aspects regarding use of the nonpenetrating captive bolt are similar to the use of a penetrating captive bolt, as previously described.

Electrical stunning—Alternating electrical current has been used for stunning species such as dogs, cattle, sheep, goats, hogs, fish and chickens.[133,134,140,177,178]

Experiments with dogs have identified a need to direct the electrical current through the brain to induce rapid loss of consciousness. In dogs, when electricity passes only between fore- and hind limbs or neck and feet, it causes the heart to fibrillate but does not induce sudden loss of consciousness.[139] For electrical stunning of any animal, an apparatus that applies electrodes to opposite sides of the head, or in another way directs electrical current immediately through the brain, is necessary to induce rapid loss of consciousness. Attachment of electrodes and animal restraint can pose problems with this form of stunning. Signs of effective electrical stunning are extension of the limbs, opisthotonos, downward rotation of the eyeballs, and tonic spasm changing to clonic spasm, with eventual muscle flaccidity.

Electrical stunning should be followed promptly by electrically induced cardiac fibrillation, exsanguination, or other appropriate methods to ensure death. Refer to the section on electrocution for additional information.

Pithing

In general, pithing is used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means. For some species, such

as frogs, with anatomic features that facilitate easy access to the central nervous system, pithing may be used as a sole means of euthanasia, but an anesthetic overdose is a more suitable method.

SPECIAL CONSIDERATIONS

EQUINE EUTHANASIA

Pentobarbital or a pentobarbital combination is the best choice for equine euthanasia. Because a large volume of solution must be injected, use of an intravenous catheter placed in the jugular vein will facilitate the procedure. To facilitate catheterization of an excitable or fractious animal, a tranquilizer such as acepromazine, or an alpha-2 adrenergic agonist can be administered, but these drugs may prolong time to loss of consciousness because of their effect on circulation and may result in varying degrees of muscular activity and agonal gasping. Opioid agonists or agonist/antagonists in conjunction with alpha-2 adrenergic agonists may further facilitate restraint.

In certain emergency circumstances, such as euthanasia of a horse with a serious injury at a racetrack, it may be difficult to restrain a dangerous horse or other large animal for intravenous injection. The animal might cause injury to itself or to bystanders before a sedative could take effect. In such cases, the animal can be given a neuromuscular blocking agent such as succinylcholine, but the animal must be euthanatized with an appropriate technique as soon as the animal can be controlled. Succinylcholine alone or without sufficient anesthetic must not be used for euthanasia.

Physical methods, including gunshot, are considered conditionally acceptable techniques for equine euthanasia. The penetrating captive bolt is acceptable with appropriate restraint.

ANIMALS INTENDED FOR HUMAN OR ANIMAL FOOD

In euthanasia of animals intended for human or animal food, chemical agents that result in tissue residues cannot be used, unless they are approved by the US Food and Drug Administration.[179] Carbon dioxide is the only chemical currently used for euthanasia of food animals (primarily swine) that does not result in tissue residues. Physical techniques are commonly used for this reason. Carcasses of animals euthanatized by barbituric acid derivatives or other chemical agents may contain potentially harmful residues. These carcasses should be disposed of in a manner that will prevent them from being consumed by human beings or animals.

Selection of a proper euthanasia technique for free-ranging wildlife must take into account the possibility of consumption of the carcass of the euthanatized animal by nontarget predatory or scavenger species. Numerous cases of toxicosis and death attributable to ingestion of pharmaceutically contaminated carcasses in predators and scavengers have been reported.[107] Proper carcass disposal must be a part of any euthanasia procedure under free-range conditions where there is potential for consumption toxicity. When carcasses are to be left in the field, a gunshot to the head, penetrating captive bolt, or injectable agents that are nontoxic (potassium chloride in combination with a nontoxic general anesthetic) should be used so that the potential for scavenger or predator toxicity is lessened.

EUTHANASIA OF NONCONVENTIONAL SPECIES: ZOO, WILD, AQUATIC, AND ECTOTHERMIC ANIMALS

Compared with objective information on companion, farm, and laboratory animals, euthanasia of species such as zoo, wild, aquatic, and ectothermic animals has been studied less, and guidelines are more limited. Irrespective of the unique or unusual features of some species, whenever it becomes necessary to euthanatize an animal, death must be induced as painlessly and quickly as possible.

When selecting a means of euthanasia for these species, factors and criteria in addition to those previously discussed must be considered. The means selected will depend on the species, size, safety aspects, location of the animals to be euthanatized, and experience of personnel. Whether the animal to be euthanatized is in the wild, in captivity, or free-roaming are major considerations. Anatomic differences must be considered. For example, amphibians, fish, reptiles, and marine mammals differ anatomically from domestic species. Veins may be difficult to locate. Some species have a carapace or other defensive anatomic adaptations (eg, quills, scales, spines). For physical methods, access to the central nervous system may be difficult because the brain may be small and difficult to locate by inexperienced persons.

Zoo Animals

For captive zoo mammals and birds with related domestic counterparts, many of the means described previously are appropriate. However, to minimize injury to persons or animals, additional precautions such as handling and physical or chemical restraint are important considerations.16

Wildlife

For wild and feral animals, many recommended means of euthanasia for captive animals are not feasible. The panel recognized there are situations involving free-ranging wildlife when euthanasia is not possible from the animal or human safety standpoint, and killing may be necessary. Conditions found in the field, although more challenging than those that are controlled, do not in any way reduce or minimize the ethical obligation of the responsible individual to reduce pain and distress to the greatest extent possible during the taking of an animal's life. Because euthanasia of wildlife is often performed by lay personnel in remote settings, guidelines are needed to assist veterinarians, wildlife biologists, and wildlife health professionals in developing humane protocols for euthanasia of wildlife.

In the case of free-ranging wildlife, personnel may not be trained in the proper use of remote anesthesia, proper delivery equipment may not be available, personnel may be working alone in remote areas where accidental exposure to potent anesthetic medications used in wildlife capture would present a risk to human safety, or approaching the animal within a practical darting distance may not be possible. In these cases, the only practical means of animal collection may be gunshot and kill trapping.[13,180-184] Under these conditions, specific methods chosen must be as age-, species-, or taxonomic/class-specific as possible. The firearm and ammunition should be appropriate for the species and purpose. Personnel should be sufficiently skilled to be accurate, and they should be experienced in the proper and safe use of firearms, complying with laws and regulations governing their possession and use.

Behavioral responses of wildlife or captive nontraditional species (zoo) in close human contact are very different from those of domestic animals. These animals are usually frightened and distressed. Thus, minimizing the amount, degree, and/or cognition of human contact during procedures that require handling is of utmost importance. Handling these animals often requires general anesthesia, which provides loss of consciousness and which relieves distress, anxiety, apprehension, and perception of pain. Even though the animal is under general anesthesia, minimizing auditory, visual, and tactile stimulation will help ensure the most stress-free euthanasia possible. With use of general anesthesia, there are more methods for euthanasia available.

A 2-stage euthanasia process involving general anesthesia, tranquilization, or use of analgesics, followed by intravenous injectable pharmaceuticals, although preferred, is often not practical. Injectable anesthetics are not always legally or readily available to those working in nuisance animal control, and the distress to the animal induced by live capture, transport to a veterinary facility, and confinement in a veterinary hospital prior to euthanasia must be considered in choosing the most humane technique for the situation at hand. Veterinarians providing support to those working with injured or live-trapped, free-ranging animals should take capture, transport, handling distress, and possible carcass consumption into consideration when asked to assist with euthanasia. Alternatives to 2-stage euthanasia using anesthesia include a squeeze cage with intraperitoneal injection of sodium pentobarbital, inhalant agents (CO_2 chamber, CO chamber), and gunshot. In cases where preeuthanasia anesthetics are not available, intraperitoneal injections of sodium pentobarbital, although slower in producing loss of consciousness, should be considered preferable over intravenous injection, if restraint will cause increased distress to the animal or danger to the operator.

Wildlife species may be encountered under a variety of situations. Euthanasia of the same species under different conditions may require different techniques. Even in a controlled setting, an extremely fractious large animal may threaten the safety of the practitioner, bystanders, and itself. When safety is in question and the fractious large animal, whether wild, feral, or domestic, is in close confinement, neuromuscular blocking agents may be used immediately prior to the use of an acceptable form of euthanasia. For this technique to be humane, the operator must ensure they will gain control over the animal and perform euthanasia before distress develops. Succinylcholine is not acceptable as a method of restraint for use in free-ranging wildlife because animals may not be retrieved rapidly enough to prevent neuromuscular blocking agent-induced respiratory distress or arrest.[185]

Diseased, Injured, or Live-Captured Wildlife or Feral Species

Euthanasia of diseased, injured, or live-trapped wildlife should be performed by qualified professionals. Certain cases of wildlife injury (eg, acute, severe trauma from automobiles) may require immediate action, and pain and suffering in the animal may be best relieved most rapidly by physical methods including

gunshot or penetrating captive bolt followed by exsanguination.

Birds

Many techniques discussed previously in these guidelines are suitable for euthanasia of captive birds accustomed to human contact. Free-ranging birds may be collected by a number of methods, including nets and live traps, with subsequent euthanasia. For collection by firearm, shotguns are recommended. The bird should be killed outright by use of ammunition loads appropriate for the species to be collected. Wounded birds should be killed quickly by appropriate techniques previously described. Large birds should be anesthetized prior to euthanasia, using general anesthetics.

Amphibians, Fish, and Reptiles

Euthanasia of ectothermic animals must take into account differences in their metabolism, respiration, and tolerance to cerebral hypoxia. In addition, it is often more difficult to ascertain when an animal is dead. Some unique aspects of euthanasia of amphibians, fishes, and reptiles have been described.[13,51,186,187]

Injectable agents—Sodium pentobarbital (60 to 100 mg/kg of body weight) can be administered intravenously, intraabdominally, or intrapleuroperitoneally in most ectothermic animals, depending on anatomic features. Subcutaneous lymph spaces may also be used in frogs and toads. Time to effect may be variable, with death occurring in up to 30 minutes.[1,187,188] Barbiturates other than pentobarbital can cause pain on injection.[189]

Clove oil—Because adequate and appropriate clinical trials have not been performed on fish to evaluate its effects, use of clove oil is not acceptable.

External or topical agents—Tricaine methane sulfonate (TMS, MS-222) may be administered by various routes to euthanatize. For fish and amphibians, this chemical may be placed in water.[190-193] Large fish may be removed from the water, a gill cover lifted, and a concentrated solution from a syringe flushed over the gills. MS 222 is acidic and in concentrations ≥ 500 mg/L should be buffered with sodium bicarbonate to saturation resulting in a solution pH of 7.0 to 7.5.105 MS 222 may also be injected into lymph spaces and pleuroperitoneal cavities.[194] These are effective but expensive means of euthanasia.

Benzocaine hydrochloride, a compound similar to TMS, may be used as a bath or in a recirculation system for euthanasia of fish[184] or amphibians.[13] Benzocaine is not water soluble and therefore is prepared as a stock solution (100 g/L), using acetone or ethanol, which may be irritating to fish tissues. In contrast, benzocaine hydrochloride is water soluble and can be used directly for anesthesia or euthanasia.[105] A concentration ≥ 250 mg/L can be used for euthanasia. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement.[104]

The anesthetic agent 2-phenoxyethanol is used at concentrations of 0.5 to 0.6 ml/L or 0.3 to 0.4 mg/L for euthanasia of fish. Death is caused by respiratory collapse. As with other agents, fish should be left in solution for 10 minutes following cessation of opercular movement.[195,196]

Inhalant agents—Many reptiles and amphibians, including chelonians, are capable of holding their breath and converting to anaerobic metabolism, and can survive long periods of anoxia (up to 27 hours for some species).[197-202] Because of this ability to tolerate anoxia, induction of anesthesia and time to loss of consciousness may be greatly prolonged when inhalants are used. Death in these species may not occur even after prolonged inhalant exposure.[203] Lizards, snakes, and fish do not hold their breath to the same extent and can be euthanatized by use of inhalant agents.

Carbon dioxide—Amphibians,[1] reptiles,[1] and fish[203-205] may be euthanatized with CO_2. Loss of consciousness develops rapidly, but exposure times required for euthanasia are prolonged. This technique is more effective in active species and those with less tendency to hold their breath.

Physical methods—Line drawings of the head of various amphibians and reptiles, with recommended locations for captive bolt or firearm penetration, are available.[13] Crocodilians and other large reptiles can also be shot through the brain.[51]

Decapitation with heavy shears or a guillotine is effective for some species that have appropriate anatomic features. It has been assumed that stopping blood supply to the brain by decapitation causes rapid loss of consciousness. Because the central nervous system of reptiles, fish, and amphibians is tolerant to hypoxic and hypotensive conditions,[13] decapitation must be followed by pithing. [188]

Two-stage euthanasia procedures—Propofol and ultrashort-acting barbiturates may be used for these species to produce rapid general anesthesia prior to final administration of euthanasia.

In zoos and clinical settings, neuromuscular blocking agents are considered acceptable for restraint of reptiles if given immediately prior to administration of a euthanatizing agent.

Most amphibians, fishes, and reptiles can be euthanatized by cranial concussion (stunning) followed by decapitation, pithing, or some other physical method.

Severing the spinal cord behind the head by pithing is an effective method of killing some ectotherms. Death may not be immediate unless both the brain and spinal cord are pithed. For these animals, pithing of the spinal cord should be followed by decapitation and pithing of the brain or by another appropriate procedure. Pithing requires dexterity and skill and should only be done by trained personnel. The pithing site in frogs is the foramen magnum, and it is identified by a slight midline skin depression posterior to the eyes with the neck flexed.[187]

Cooling—It has been suggested that, when using physical methods of euthanasia in ectothermic species, cooling to 4 C will decrease metabolism and facilitate handling, but there is no evidence that whole body cooling reduces pain or is clinically efficacious.[206] Local cooling in frogs does reduce nociception, and this may be partly opioid mediated. [207] Immobilization of reptiles by cooling is considered inappropriate and inhumane even if combined with other physical or chemical methods of euthanasia. Snakes and turtles, immobilized by cooling, have been killed by subsequent freezing. This method is not recommended.[13] Formation of ice crystals on the skin and in tissues of an animal may cause pain or distress. Quick freezing of deeply anesthetized animals is acceptable.[208]

Marine Mammals

Barbiturates or potent opioids (eg, etorphine hydrochloride [M 99] and carfentanil) are the agents of choice for euthanasia of marine mammals,[209] although it is recognized their use is not always possible and can be potentially dangerous to personnel. An accurately placed gunshot may also be a conditionally acceptable method of euthanasia for some species and sizes of stranded marine mammals.[51,209,210]

For stranded whales or other large cetaceans or pinnipeds, succinylcholine chloride in conjunction with potassium chloride, administered intravenously or intraperitoneally, has been used.[211] This method, which is not an acceptable method of euthanasia as defined in these guidelines, leads to complete paralysis of the respiratory musculature and eventual death attributable to hypoxemia.[209] This method may be more humane than allowing the stranded animal to suffocate over a period of hours or days if no other options are available.

EUTHANASIA OF ANIMALS RAISED FOR FUR PRODUCTION

Animals raised for fur are usually euthanatized individually at the location where they are raised. Although any handling of these species constitutes a stress, it is possible to minimize this by euthanatizing animals in or near their cages. For the procedures described below, please refer to previous sections for more detailed discussion.

Carbon monoxide—For smaller species, CO appears to be an adequate method for euthanasia. Compressed CO is delivered from a tank into an enclosed cage that can be moved adjacent to holding cages. Using the apparatus outside reduces the risk to humans; however, people using this method should still be made aware of the dangers of CO. Animals introduced into a chamber containing 4% CO lost consciousness in 64 ± 14 seconds and were dead within 215 ± 45 seconds.[80] In a study involving electroencephalography of mink being euthanatized with 3.5% CO, the mink were comatose in 21 ± 7 seconds.212 Only 1 animal should be introduced into the chamber at a time, and death should be confirmed in each case.

Carbon dioxide—Administration of CO_2 is also a good euthanasia method for smaller species and is less dangerous than CO for personnel operating the system. When exposed to 100% CO_2, mink lost consciousness in 19 ± 4 seconds and were dead within 153 ± 10 seconds. When 70% CO_2 was used with 30% O_2, mink were unconscious in 28 seconds, but they were not dead after a 15-minute exposure.[80] Therefore, if animals are first stunned by 70% CO_2, they should be killed by exposure to 100% CO_2 or by some other means. As with carbon monoxide, only one animal should be introduced into the chamber at a time.

Barbiturates—Barbiturate overdose is an acceptable procedure for euthanasia of many species of animals raised for fur. The drug is injected intraperitoneally and the animal slowly loses consciousness. It is important that the death of each animal be confirmed following barbiturate injection. Barbiturates will contaminate the carcass; therefore the skinned carcass cannot be used for animal food.

Electrocution—Electrocution has been used for killing foxes and mink.[135] The electric current must pass through the brain to induce loss of consciousness before electricity is passed through the rest of the body. Electrical stunning should be followed by euthanasia, using some other technique. Cervical dislocation has been used in mink and other small animals and should be done within 20 seconds of electrical stunning.213

Use of a nose-to-tail or nose-to-foot method[135] alone may kill the animal by inducing cardiac fibrillation, but the animal may be conscious for a period of time before death. Therefore, these techniques are unacceptable.

PRENATAL AND NEONATAL EUTHANASIA

When ovarian hysterectomies are performed, euthanasia of feti should be accomplished as soon as possible after removal from the dam. Neonatal animals are relatively resistant to hypoxia.[44,214]

MASS EUTHANASIA

Under unusual conditions, such as disease eradication and natural disasters, euthanasia options may be limited. In these situations, the most appropriate technique that minimizes human and animal health concerns must be used. These options include, but are not limited to, CO_2 and physical methods such as gunshot, penetrating captive bolt, and cervical dislocation.

POSTFACE

These guidelines summarize contemporary scientific knowledge on euthanasia in animals and call attention to the lack of scientific reports assessing pain, discomfort, and distress in animals being euthanatized. Many reports on various methods of euthanasia are either anecdotal, testimonial narratives, or unsubstantiated opinions and are, therefore, not cited in these guidelines. The panel strongly endorsed the need for well-designed experiments to more fully determine the extent to which each procedure meets the criteria used for judging methods of euthanasia.

Each means of euthanasia has advantages and disadvantages. It is unlikely that, for each situation, any means will meet all desirable criteria. It is also impractical for these guidelines to address every potential circumstance in which animals are to be euthanatized. Therefore, the use of professional judgment is imperative.

Failure to list or recommend a means of euthanasia in these guidelines does not categorically condemn its use. There may occasionally be special circumstances or situations in which other means may be acceptable. For research animals, these exceptions should be carefully considered by the attending veterinarian and the Institutional Animal Care and Use Committee. In other settings, professional judgment should be used.

The panel discouraged the use of unapproved products for euthanasia, unless the product has a clearly understood mechanism of action and pharmacokinetics, and studies published in the literature that scientifically verify and justify its use. Those responsible for euthanasia decisions have a critically important responsibility to carefully assess any new technique, method, or device, using the panel's criteria. In the absence of definitive proof or reasonable expectation, the best interest of the animal should guide the decision process.

References cited in these guidelines do not represent a comprehensive bibliography on all methods of euthanasia. Persons interested in additional information on a particular aspect of animal euthanasia are encouraged to contact the Animal Welfare Information Center, National Agricultural Library, 10301 Baltimore Blvd, Beltsville, MD 20705.

The AVMA is fully committed to the concept that, whenever it becomes necessary to kill any animal for any reason whatsoever, death should be induced as painlessly and quickly as possible. It was the Panel's charge to develop workable guidelines for veterinarians needing to address this problem, and it is the AVMA's sincere desire that these guidelines be used conscientiously by all animal care providers. We consider these guidelines to be a work in progress with new editions warranted as results of more scientific studies are published.

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Appendix 1—Agents and methods of euthanasia by species

Agents and methods of euthanasia by species (refer to Appendix 4 for unacceptable agents and methods.)

Species	Acceptable* (refer to Appendix 2 and text for details)	Conditionally acceptable† (refer to Appendix 3 and text for details)
Amphibians	Barbiturates, inhalant anesthetics (in appropriate species), CO_2, CO, tricaine methane sulfonate (TMS, MS 222), benzocaine hydrochloride, double pithing	Penetrating captive bolt, gunshot, stunning and decapitation, decapitation and pithing
Birds	Barbiturates, inhalant anesthetics, CO_2, CO, gunshot (free-ranging only)	N_2, Ar, cervical dislocation, decapitation, thoracic compression (small, free-ranging only), maceration (chicks, poults, and pipped eggs only)
Cats	Barbiturates, inhalant anesthetics, CO_2, CO, potassium chloride in conjunction with general anesthesia	N_2, Ar
Dogs	Barbiturates, inhalant anesthetics, CO_2, CO, potassium chloride in conjunction with general anesthesia	N_2, Ar, penetrating captive bolt, electrocution
Fish	Barbiturates, inhalant anesthetics, CO_2, tricaine methane sulfonate (TMS, MS 222), benzocaine hydrochloride, 2-phenoxyethanol	Decapitation and pithing, stunning and decapitation/pithing
Horses	Barbiturates, potassium chloride in conjunction with general anesthesia, penetrating captive bolt	Chloral hydrate (IV, after sedation), gunshot, electrocution
Marine mammals	Barbiturates, etorphine hydrochloride	Gunshot (cetaceans < 4 meters long)
Mink, fox, and other mammals produced for fur	Barbiturates, inhalant anesthetics, CO_2 (mink require high concentrations for euthanasia without supplemental agents), CO, potassium chloride in conjunction with general anesthesia	N_2, Ar, electrocution followed by cervical dislocation
Nonhuman primates	Barbiturates	Inhalant anesthetics, CO_2, CO, N_2, Ar
Rabbits	Barbiturates, inhalant anesthetics, CO_2, CO, potassium chloride in conjunction with general anesthesia	N_2, Ar, cervical dislocation (< 1 kg), decapitation, penetrating captive bolt
Reptiles	Barbiturates, inhalant anesthetics (in appropriate species), CO_2 (in appropriate species)	Penetrating captive bolt, gunshot, decapitation and pithing, stunning and decapitation

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Species	Acceptable* (refer to Appendix 2 and text for details)	Conditionally acceptable† (refer to Appendix 3 and text for details)
Rodents and other small mammals	Barbiturates, inhalant anesthetics, CO_2, CO, potassium chloride in conjunction with general anesthesia, microwave irradiation	Methoxyflurane, ether, N_2, Ar, cervical dislocation (rats < 200 g), decapitation
Ruminants	Barbiturates, potassium chloride in conjunction with general anesthesia, penetrating captive bolt	Chloral hydrate (IV, after sedation), gunshot, electrocution
Swine	Barbiturates, CO_2, potassium chloride in conjunction with general anesthesia, penetrating captive bolt	Inhalant anesthetics, CO, chloral hydrate (IV, after sedation), gunshot, electrocution, blow to the head (< 3 weeks of age)
Zoo animals	Barbiturates, inhalant anesthetics, CO_2, CO, potassium chloride in conjunction with general anesthesia	N_2, Ar, penetrating captive bolt, gunshot
Free-ranging wildlife	Barbiturates IV or IP, inhalant anesthetics, potassium chloride in conjunction with general anesthesia	CO_2, CO, N_2, Ar, penetrating captive bolt, gunshot, kill traps (scientifically tested)

* Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia.
†Conditionally acceptable methods are those that by the nature of the technique or because of greater potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in the scientific literature.

Appendix 2—Acceptable agents and methods of euthanasia

Acceptable agents and methods of euthanasia—characteristics and modes of action (refer to text for details)

Agent	Classification	Mode of action	Rapidity	Ease of performance	Safety for personnel	Species suitability	Efficacy and comments
Barbiturates	Hypoxia attributable to depression of vital centers	Direct depression of cerebral cortex, subcortical structures, and vital centers; direct depression of heart muscle	Rapid onset of anesthesia	Animal must be restrained; personnel must be skilled to perform IV injection	Safe except human abuse potential; DEA-controlled substance	Most species	Highly effective when appropriately administered; acceptable IP in small animals and IV
Benzocaine hydrochloride	Hypoxia attributable to depression of vital centers	Depression of CNS	Very rapid, depending on dose	Easily used	Safe	Fish, amphibians	Effective but expensive
Carbon dioxide (bottled gas only)	Hypoxia attributable to depression of vital centers	Direct depression of cerebral cortex, subcortical structures, and vital centers; direct depression of heart muscle	Moderately rapid	Used in closed container	Minimal hazard	Small laboratory animals, birds, cats, small dogs, rabbits, mink (high concentrations required), zoo animals, amphibians, fish, some reptiles, swine	Effective, but time required may be prolonged in immature and neonatal animals
Carbon monoxide (bottled gas only)	Hypoxia	Combines with hemoglobin, preventing its combination with oxygen	Moderate onset time, but insidious so animal is unaware of onset	Requires appropriately maintained equipment	Extremely hazardous, toxic, and difficult to detect	Most small species including dogs, cats, rodents, mink, chinchillas, birds, reptiles, amphibians, zoo animals, rabbits	Effective; acceptable only when equipment is properly designed and operated

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Agent	Classification	Mode of action	Rapidity	Ease of performance	Safety for personnel	Species suitability	Efficacy and comments
Inhalant anesthetics	Hypoxia attributable to depression of vital centers	Direct depression of cerebral cortex, subcortical structures, and vital centers	Moderately rapid onset of anesthesia, excitation may develop during induction	Easily performed with closed container; can be administered to large animals by means of a mask	Must be properly scavenged or vented to minimize exposure to personnel	Some amphibians, birds, cats, dogs, furbearing animals, rabbits, some reptiles, rodents and other small mammals, zoo animals, fish, free-ranging wildlife	Highly effective provided that subject is sufficiently exposed; either is conditionally acceptable
Microwave irradiation	Brain enzyme inactivation	Direct inactivation of brain enzymes by rapid heating of brain	Very rapid	Requires training and highly specialized equipment	Safe	Mice, rats	Highly effective for special needs
Penetrating captive bolt	Physical damage to brain	Direct concussion of brain tissue	Rapid	Requires skill, adequate restraint, and proper placement of captive bolt	Safe	Horses, ruminants, swine	Instant loss of consciousness, but motor activity may continue
2-Phenoxyethanol	Hypoxia attributable to depression of vital centers	Depression of CNS	Very rapid, depending on dose	Easily used	Safe	Fish	Effective but expensive
Potassium chloride (intracardially or intravenously in conjunction with general anesthesia only)	Hypoxia	Direct depression of cerebral cortex, subcortical structures, and vital centers secondary to cardiac arrest.	Rapid	Requires training and specialized equipment for remote injection anesthesia, and ability to give IV injection of potassium chloride	Anesthetics may be hazardous with accidental human exposure	Most species	Highly effective, some clonic muscle spasms may be observed
Tricaine methane sulfonate (TMS, MS 222)	Hypoxia attributable to depression of vital centers	Depression of CNS	Very rapid, depending on dose	Easily used	Safe	Fish, amphibians	Effective but expensive

Appendix 3—Conditionally acceptable agents and methods of euthanasia

Conditionally acceptable agents and methods of euthanasia—characteristics and modes of action (refer to text for details)

Agent	Classification	Mode of action	Rapidity	Ease of performance	Safety for personnel	Species suitability	Efficacy and comments
Blow to the head	Physical damage to brain	Direct concussion of brain tissue	Rapid	Requires skill, adequate restraint, and appropriate force	Safe	Young pigs <3 weeks old	Must be properly applied to be humane and effective
Carbon dioxide (bottled gas only)	Hypoxia due to depression of vital centers	Direct depression of cerebral cortex, subcortical structures and vital centers; direct depression of heart muscle	Moderately rapid	Used in closed container	Minimal hazard	Nonhuman primates, free-ranging wildlife	Effective, but time required may be prolonged in immature and neonatal animals
Carbon monoxide (bottled gas only)	Hypoxia	Combines with hemoglobin, preventing its combination with oxygen	Moderate onset time, but insidious so animal is unaware of onset	Requires appropriately maintained equipment	Extremely hazardous, toxic, and difficult to detect	Nonhuman primates, free-ranging wildlife	Effective; acceptable only when equipment is properly designed and operated
Cervical dislocation	Hypoxia due to disruption of vital centers	Direct depression of brain	Moderately rapid	Requires training and skill	Safe	Poultry, birds, laboratory mice, rats (< 200 g), rabbits (< 1 kg)	Irreversible; violent muscle contractions can occur after cervical dislocation
Chloral hydrate	Hypoxia from depression of respiratory center	Direct depression of brain	Rapid	Personnel must be skilled to perform IV injection	Safe	Horses, ruminants, swine	Animals should be sedated prior to administration

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Agent	Classification	Mode of action	Rapidity	Ease of performance	Safety for personnel	Species suitability	Efficacy and comments
Decapitation	Hypoxia due to disruption of vital centers	Direct depression of brain	Rapid	Requires training and skill	Guillotine poses potential employee injury hazard	Laboratory rodents; small rabbits; birds; some fish, amphibians, and reptiles (latter 3 with pithing)	Irreversible; violent muscle contraction can occur after decapitation
Electrocution	Hypoxia	Direct depression of brain and cardiac fibrillation	Can be rapid	Not easily performed in all instances	Hazardous to personnel	Used primarily in sheep, swine, foxes, mink (with cervical dislocation), ruminants, animals > 5 kg	Violent muscle contractions occur at same time as loss of consciousness
Gunshot	Hypoxia due to disruption of vital centers	Direct concussion of brain tissue	Rapid	Requires skill and appropriate firearm	May be dangerous	Large domestic and zoo animals, reptiles, amphibians, wildlife, cetaceans (< 4 meters long)	Instant loss of consciousness, but motor activity may continue
Inhalant anesthetics	Hypoxia due to disruption of vital centers	Direct depression of cerebral cortex, subcortical structures, and vital centers	Moderately rapid onset of anesthesia; excitation may develop during induction	Easily performed with closed container; can be administered to large animals by means of a mask	Must be properly scavenged or vented to minimize exposure to personnel; ether has explosive potential and exposure to ether may be stressful	Nonhuman primates, swine; ether is conditionally acceptable for rodents and small mammals; methoxyflurane is conditionally acceptable for rodents and small mammals	Highly effective provided that subject is sufficiently exposed

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Agent	Classification	Mode of action	Rapidity	Ease of performance	Safety for personnel	Species suitability	Efficacy and comments
Nitrogen, argon	Hypoxia	Reduces partial pressure of oxygen available to blood	Rapid	Used in closed chamber with rapid filling	Safe if used with ventilation	Cats, small dogs, birds, rodents, rabbits, other small species, mink, zoo animals, nonhuman primates, free-ranging wildlife	Effective except in young and neonates; an effective agent, but other methods are preferable
Penetrating captive bolt	Physical damage to brain	Direct concussion of brain tissue	Rapid	Requires skill, adequate restraint and proper placement of captive bolt	Safe	Dogs, rabbits, zoo animals, reptiles, amphibians, free-ranging wildlife	Instant loss of consciousness but motor activity may continue
Pithing	Hypoxia due to disruption of vital centers, physical damage to brain	Trauma of brain and spinal cord tissue	Rapid	Easily performed but requires skill	Safe	Some ectotherms	Effective, but death not immediate unless brain and spinal cord are pithed
Thoracic compression	Hypoxia and cardiac arrest	Physical interference with cardiac and respiratory function	Moderately rapid	Requires training	Safe	Small- to medium-sized free-ranging birds	Apparently effective
<u>Maceration</u>	<u>Physical damage to brain</u>	<u>Direct concussion of brain tissue</u>	<u>Rapid</u>	<u>Easily performed with properly designed, commercially available equipment</u>	<u>Safe</u>	<u>Newly hatched chicks and poults, and pipped eggs only</u>	<u>Effective when equipment is properly designed and operated</u>

Appendix 4—Some unacceptable agents and methods of euthanasia

Some unacceptable agents and methods of euthanasia (refer to text for details)

Agent or method	Comments
Air embolism	Air embolism may be accompanied by convulsions, opisthotonos, and vocalization. If used, it should be done only in anesthetized animals.
Blow to the head	Unacceptable for most species.
Burning	Chemical or thermal burning of an animal is not an acceptable method of euthanasia.
Chloral hydrate	Unacceptable in dogs, cats, and small mammals.
Chloroform	Chloroform is a known hepatotoxin and suspected carcinogen and, therefore, is extremely hazardous to personnel.
Cyanide	Cyanide poses an extreme danger to personnel and the manner of death is aesthetically objectionable.
Decompression	Decompression is unacceptable for euthanasia because of numerous disadvantages. (1) Many chambers are designed to produce decompression at a rate 15 to 60 times faster than that recommended as optimum for animals, resulting in pain and distress attributable to expanding gases trapped in body cavities. (2) Immature animals are tolerant of hypoxia, and longer periods of decompression are required before respiration ceases. (3) Accidental recompression, with recovery of injured animals, can occur. (4) Bleeding, vomiting, convulsions, urination, and defecation, which are aesthetically unpleasant, may develop in unconscious animals.
Drowning	Drowning is not a means of euthanasia and is inhumane.
Exsanguination	Because of the anxiety associated with extreme hypovolemia, exsanguination should be done only in sedated, stunned, or anesthetized animals.
Formalin	Direct immersion of an animal into formalin, as a means of euthanasia, is inhumane.
Household products and solvents	Acetone, quaternary compounds (including CCl_4), laxatives, clove oil, dimethylketone, quaternary ammonium products*, antacids, and other commercial and household products or solvents are not acceptable agents for euthanasia.

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Agent or method	Comments
Hypothermia	Hypothermia is not an appropriate method of euthanasia.
Neuromuscular blocking agents (nicotine, magnesium sulfate, potassiumchloride, all curariform agents)	When used alone, these drugs all cause respiratory arrest before loss of consciousness, so the animal may perceive pain and distress after it is immobilized.
Rapid freezing	Rapid freezing as a sole means of euthanasia is not considered to be humane. If used, animals should be anesthetized prior to freezing.
Smothering	Smothering of chicks or poults in bags or containers is not acceptable.
Strychnine	Strychnine causes violent convulsions and painful muscle contractions.
Stunning	Stunning may render an animal unconscious, but it is not a method of euthanasia (except for neonatal animals with thin craniums). If used, it must be immediately followed by a method that ensures death.
Tricaine methane sulfonate (TMS, MS 222)	Should not be used for euthanasia of animals intended as food.
*Roccal D Plus, Pharmacia & Upjohn, Kalamazoo, Michigan	